REMARKS

Sequence Listing Requirements

The Sequence Listing has been objected to as containing errors in the computer readable form. Applicants request this issue be held in abeyance for the time being. A replacement disk will be prepared and submitted within the six month statutory time for responding.

In Item 3, the Examiner indicates that Applicants incorrectly directed that the Sequence Listing be inserted after the Abstract of the Disclosure. The Examiner suggested that the Sequence Listing should be inserted immediately before the claims. Applicants respectfully note that the recent revisions of the Sequence Listing Rules now require the Sequence Listing to be inserted after the Abstract of the Disclosure on independently numbered pages, as submitted on March 9, 1999.

Objections to the claims

Claim 9 have been objected to for containing a typographical error in the word "said." Claim 9 has been amended to correct the typographical error. As such, withdrawal of the objection is respectfully requested.

Rejections under 35 U.S.C.§112, first paragraph

On page 13, Item f) of the Office Action, the Examiner rejected claims 6 and 8-10 under 35 U.S.C.§112, first paragraph for lack of enablement. More specifically, the Examiner asserts that the use of primers to obtain an amplified product is essential to the invention. Applicants traverse this rejection and withdrawal thereof is respectfully requested.

Step e) of claim 6 recites, "amplifying said conjugated oligonucleotides." It would be well known to one skilled in the art that "amplifying" oligonucleotides inherently means adding primers etc., as necessary. As detailed on page 5, last paragraph of the specification, a preferred amplification technique is PCR. The individualized steps associated with PCR or other forms of amplification are well known and would be readily known to be intended simply by reciting "amplifying." As such, claims 6 and 8-10 are enabled as currently written. Withdrawal of the rejection is, therefore respectfully requested.

Rejections under 35 U.S.C.§112, second paragraph

Claim 6 has been rejected under 35 U.S.C.§112, second paragraph as being indefinite. More specifically, the Examiner asserts that claim 6 is indefinite for the following reasons.

a) The Examiner asserts that claim 6 fails to recite that the oligonucleotides are cross linked. Claim 6 is further asserted to be unclear in the recitation that the second and third antibodies "become cross linked" and because the components and reactions for the amplification and cross linking reactions are not well defined.

b) In Item 5 of the Office Action, the Examiner asserts that the term "cross link" is indefinite in that it implies both specific and non-specific binding reactions. The Examiner suggests that the term "ligate" be used instead of "cross link" to be indicative of a specific reaction.

c) In Item 6 of the Office Action, the Examiner suggests amending claim 6 to replace steps b) and d) with "washing off excess sample" and "washing off excess solution," respectively.

With regard to point a) above, claim 6 has been amended to more clearly recite that "said oligonucleotides conjugate to each other when said second and third antibody are both bound to said antigen."

With regard to point b) above, claim 6 has been amended as indicated above to replace the term "cross link," or derivations thereof, with "conjugate" and appropriate derivations of "conjugate." Support for this amendment may be found in the final two lines of page 4 of the specification. Applicants believe the term "conjugate" provides the required clarity to claim without the undue limitation which may be associated with using the term "ligate." As indicated on

page 5, lines 1-15, of the specification, the "conjugation" of the oligonucleotides, while specific, is not necessarily a "ligation." As such, it is believed that "conjugate" is a more appropriate term.

Finally, claim 6 has been additionally amended pursuant to the suggestion of the Examiner in point c) above.

In Items 25 a) and b) the Examiner rejected claim 6 for recitation of "said, same antigen" and "said cross linked oligonucleotide." Claim 6 has been amended, as indicated above, to address these issues.

In Item 25 c), the Examiner rejected claims 8-10 for recitation of "complementary oligonucleotide to the crosslinkable oligonucleotide before step d)." The Examiner indicates that it is unclear to what extent the complementary oligonucleotide is complementary to the oligonucleotides bound to the second and third antibodies or if the complementary oligonucleotide participates in the crosslinking reactions. The Examiner further notes that if the complementary oligonucleotide is complementary to both of the oligonucleotides on the second and third antibodies addition of a ligase, as recited in claim 10, is confusing because the complementary oligonucleotide and the oligonucleotides of the second and third antibodies would not be expected to be in a conformation suitable for ligation to each other.

With regard to how and from where signal is produced with the present invention, attention is drawn to the specification on page 5, 3rd paragraph, wherein it is indicated that "only in those cases where the antibodies are brought close enough through binding of the same antigen molecule can the oligonucleotide be ligated [or conjugated]." Further, the "ligated [or conjugated] molecules subsequently serve as templates for nucleic acid amplification reactions." The specification further teaches on page 5, first full paragraph, numerous detection systems which may be utilized with the present invention.

With regard to the Examiner specific confusion regarding the complementary strands in claims 8-10, as discussed on page 5, first paragraph and the Example of the specification, the complementary strand of claim 8 is a "help" oligonucleotide for joining the two oligonucleotides bound to the antibodies. Claim 10 has been additionally amended to depend only from claim 8. In claim 9, the oligonucleotides bound to the antibodies are complementary to each other and as such no ligase is needed.

Claims 8 and 10 have been rejected as being unclear in the recitation of "adding a ligase before step d)." Claim 10 has been amended to depend only from claim 8.

Finally, the Examiner notes that the specification teaches on page 7, the use of complementary oligonucleotides, but that the exemplified sequences are not complementary. Applicants respectfully note that the

five 3' most triplets of Oligo 1 and the five 5' most triplets of Oligo 2 are complementary with the complementary "help" sequence of page 7.

Applicants believe these comments and amendments address the confusion of the Examiner and overcome the rejections of the claims as being unclear. As such, withdrawal of the rejections is respectfully requested.

Rejections under 35 U.S.C.§102(e)

Claims 1-5 remain rejected under 35 U.S.C.§102(e) as being anticipated by Urdea et al.

Claims 1 and 3-5 remain rejected under 35 U.S.C.§102(e) as being anticipated by Birkenmeyer et al. With regard to both Urdea et al. and Birkenmeyer et al., the Examiner asserts that the argued novel signal production of the present invention is not recited in the claims, as presented on March 9, 1999. Applicants traverse this rejection and withdrawal thereof is respectfully requested.

The claims have been amended to clearly define the signal as being generated from the conjugation and amplification of the oligonucleotides on the second and third affinity reagents when the second and third affinity reagents are closely bound to the same antigen. As such, the present invention is clearly distinguished from Urdea et al. and Birkenmeyer et al. Withdrawal of the rejection is therefore respectfully requested.

Rejections under 35 U.S.C. §103

Claims 1 and 3-5 have been rejected under 35 U.S.C.§103 as being obvious over Nickerson et al.; Delahunty et al. or Kwok et al. The Examiner asserts that the present invention is obvious in that it is not limited to the use of antibodies to detect protein antigens. Applicants traverse this rejection and withdrawal thereof is respectfully requested.

The present invention is drawn to an assay method and kit therefor for detecting a specific antigen wherein a macromolecule (antigen) will be detected only when two affinity reagents are closely bound to the same macromolecule. As clearly indicated on page 6, second full paragraph, "the present invention is not restricted to detection of any special kind of macromolecule." "The only criterion it [the macromolecule] has to fulfil is that it must be able to simultaneously bind three antibodies/affinity reagents."

Claims 1 and 3-5 have been amended to recite that a signal is generated by amplification of the oligonucleotides in the kit only when the second and third affinity reagents are closely bound to the macromolecule. There is no disclosure or suggestion in the prior art of the detection means and signal generation of the present kit. As such, the presently claimed kit, which will function to generate a signal by amplification of the oligonucleotides only when the second and third affinity reagents are closely bound to the macromolecule is

clearly not obvious over Nickerson et al.; Delahunty et al. or Kwok et al. Withdrawal of the rejection is therefore respectfully requested.

Claims 1-2 and 6 have been rejected under 35 U.S.C.§103 as being obvious over Lee et al. in view of Dattagupta et al. Lee et al. is asserted to disclose a three site or more immunoassay with antigens having three or more distinct epitopes. Lee et al. is further asserted to teach that using multiple affinity reagents will increase sensitivity of an antigen binding assay and reduce cross reactivity of antigen analogs etc. Dattagupta et al. is asserted to teach the covalent coupling of nucleic acid to antibodies and the use of the nucleic acid labeled antibodies as a probe in an immunoassay. Applicants traverse this rejection and withdrawal thereof is respectfully requested.

As noted by the Examiner, Lee et al. teach the use of multiple affinity reagents and Dattagupta et al. teach the use of oligonucleotides conjugated to antibodies in immunoassay and both approaches are intended to improve sensitivity. However, combining these references fails to teach the present invention.

The present invention is not simply the use of multiple probes as taught by Lee et al. or the use olignucleotides bound to antibodies as a probe. If Lee et al. is combined with Dattagupta et al. the teaching which results is the use of multiple antibodies having

oligonucleotides as a detection mechanism, i.e. Lee et al. combined with Dattagupta et al. teaches the use of multiple, but separate, independent probes, in the form of amplified DNA. However this teaching is not the present invention.

The present invention is based on the conjugation between oligonucleotides located on different affinity reagents/antibodies wherein the conjugated oligonucleotides together then serve as a template in the amplification reaction. The signal generation of the present invention is not based on the separate, independent binding of multiple probes as with Lee et al. in combination with Dattagupta et al. Rather the present invention requires the interaction between two probes to generate a signal. The teachings of Lee et al. and Dattagupta et al. are effectively discussed in the introduction section of the specification. See for example the discussion regarding Cantor et al. on page 1. Combining the teachings of Lee et al. and Dattagupta et al., results in an assay method which has the drawbacks discussed in the specification of unspecific background signal.

There is no suggestion in either Lee et al. or Dattagupta et al. generating a signal only when two affinity reagents bind in close enough proximity to the antigen to enable the formation of an amplifiable molecule. As further discussed in the specification, the present invention has increased sensitivity and reduced background

signals. As such, the present invention is clearly not obvious over Lee et al. in combination with Dattagupta et al.

Claims 3-5 and 8 have been rejected under 35 U.S.C§103 as being obvious over Lee et al., in view of Dattagupta et al. and in further view of Ciechanover et al. Further to the rejection above, regarding Lee et al. in combination with Dattagupta et al., Ciechanover et al. is asserted to teach antibodies ligated to detectably labeled with DNA and the ligation and amplification of nucleic acid targets. Applicants traverse this rejection and withdrawal thereof is respectfully requested.

As discussed above, the present assay method and kit will only generate a signal if the second and third affinity reagents are bound closely on the macromolecule so as to allow conjugation of the oligonucleotides bound to the affinity reagents. Lee et al. in combination with Dattagupta et al. merely teach the use of multiple independent probes labeled with DNA. Cienchanover et al. merely teach the amplification of oligonucleotides bound to antibodies using a ligase reaction. There is no suggestion in Cienchanover et al. of forming an amplifiable template by requiring two separate antibodies to both bind and the respective oligonucleotides on the antibodies to become conjugated. As such, the present invention is not achieved by

further combining Cienchanover et al. with Lee et al. and Dattagupta et al.

Claims 1-4 have been newly rejected under 35 U.S.C.§103 as being obvious over Hendrickson et al. The Examiner asserts that Hendrickson et al. differs from the present invention only in failing to compile the reagents of the present invention in a kit form and that such compilation into a kit would be obvious. Applicants traverse this rejection and withdrawal thereof is respectfully requested.

Hendrickson et al. is discussed on page 2 of the specification. As indicated on page 2, Hendrickson et al. merely disclose a specific means of covalently linking DNA to an antibody. There is no suggestion in Henderickson et al. of the present kit which requires the conjugation of oligonucleotides on separately but closely bound affinity reagents to generate a signal. As such, the present invention is clearly not obvious over Hendrickson et al.

As the above-presented amendments and remarks address and overcome the rejections of the Examiner, withdrawal of the rejections and reconsideration and allowance of the claims are respectfully requested. Should the Examiner have any questions regarding the present application, she is requested to contact MaryAnne Liotta, PhD (Reg. No. 40,069) in the Washington DC area, at (703) 205-8000.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. §§1.16 or 1.17; particularly, extension of time fees.

Respectfully submitted,

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